A novel method of measuring human lymphatic pumping using indocyanine green fluorescence lymphography

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Objectives: **Lymph transportation through the body is partly controlled by the intrinsic pumping of lymphatic vessels. Although an understanding of this process is important for medical application, little is currently known because it is difficult to measure. Here, we introduce an easy, safe, and cost-effective technique for measuring lymphatic pumping in leg superficial lymphatic vessels. Readings obtained with this technique were compared with values obtained with dynamic lymphoscintigraphy. Differences in lymphatic pumping between healthy volunteers and patients with lymphedema were also investigated.**

Methods: **Indocyanine green (ICG) fluorescence lymphography was performed by subcutaneously injecting 0.3 mL of ICG (0.5%) into the dorsum of the foot. Real-time fluorescence images of lymph propulsion were obtained with an infrared-light camera system with the individual supine or sitting. A custom-made transparent sphygmomanometer cuff was wrapped around the lower leg and connected to a standard mercury sphygmomanometer. The cuff was inflated to 60 mm Hg and then gradually deflated at 5-minute intervals to lower the pressure by 10-mm Hg steps until the fluorescence contrast agent exceeded the upper border of the cuff, indicating that the lymphatic contraction had overcome the cuff pressure. Lymph pumping pressure (P_{pump}) was defined as the value of the cuff pressure when the contrast agent exceeded the upper border of the cuff. We measured P_{pump} among healthy volunteers who maintained a supine position and compared these values with measurements obtained from lymphoscintigraphy. P_{pump} values while sitting were also compared between 30 legs from healthy volunteers and 30 legs from lymphedematous patients.**

Results: **Among healthy, supine participants, P_{pump} was 25.2 ± 16.7 mm Hg (mean ± standard deviation [SD]) when measured by ICG fluorescence lymphography. These values were significantly correlated with values taken using dynamic lymphoscintigraphy ($r^2 = 0.54$, $p < .01$), while 2 SDs of the mean were approximately 20 mm Hg, suggesting a substantial disagreement between the two methods (Bland-Altman plots). In the comparison of seated meaurements, readings for healthy participants ($P_{pump} = 29.3 \pm 16.0$) were higher than those for lymphedematous participants (13.2 ± 14.9).**

Conclusion: **ICG fluorescence is an accurate—as well as a safe, easy, and economical—method of measuring lymphatic pumping. Therefore, it may develop as a vital tool for diagnosing lymphatic malfunctions even when they are only in their formative stages. Studies that use this technique may increase our knowledge of the lymphatic system as a whole, allowing us to develop better treatments for lymphatic disorders. (J Vasc Surg 2010;52:946-52.)**

The lymphatic transport system is important in a variety of physiologic processes, including immune reactions, lipid absorption, and maintenance of body fluid and macromolecule balances. One component of this system is lymphatics, the channels responsible for transporting lymph from the body back to the bloodstream. It has been proposed that the intrinsic contraction of lymphatics—a process known as lymphatic pumping—is one of the major mechanisms responsible for propelling lymph to the central lymphatic systems, and decreases in lymphatic pumping may cause lymph stasis, or lymphedema. Normal lymphatic functioning is vital for maintaining circulatory and immunologic health.[1,2]

Since the first observations of contractions in the lymphatic vessels, lymphatic pumping has been thought to propel lymph centripetally. The first observations of this activity were reported by Kinmonth and Taylor in 1956 after they observed contractions in a human thoracic duct during surgery.[3,4] Several studies over the next decade reported additional evidence of lymphatic contraction,[5,6] but it was not until 1974 that intralymphatic pressure was measured for the first time in a human leg.[7] To do this, researchers introduced a pressure transducer by cannula

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This work was supported by Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science (JSPS) and Linking Mechanism of Research Results to Practical Application from Japan Science and Technology Agency (JST).

Competition of interest: none.

Presented at the Twenty-second Annual Meeting of the American Venous Forum, Amelia Island, Fla, Feb 10-13, 2010.

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The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a competition of interest.

0741-5214/$36.00

doi:10.1016/j.jvs.2010.04.067

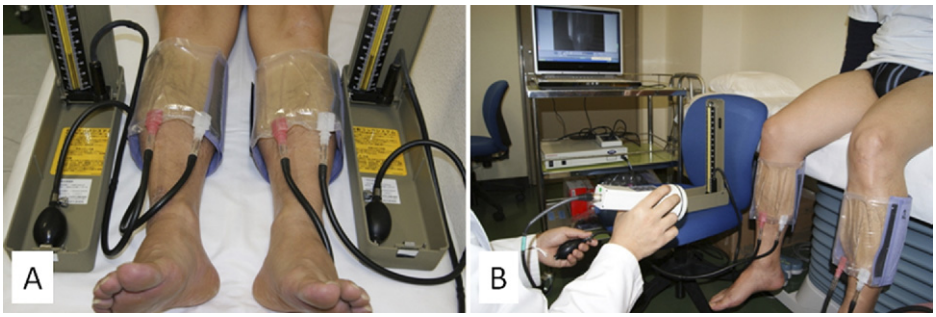

Fig 1. Measurement of lymphatic pumping in the lower leg wrapped with a custom-made transparent sphygmomanometer cuff with volunteers **(A)** supine or **(B)** sitting.

directly into the lumen of lymph vessels. Pressure ranged from 1 to 40 mm Hg. Additional research indicated that the systolic end pressure in superficial lymphatics, when the lymph flow was obstructed, was 44.7 ± 18.9 mm Hg if individuals were standing or 37.9 ± 12.9 mm Hg if supine.[8] Other groups using similar methodologies also reported that the peak pressure of leg superficial lymphatics was about 30 to 40 mm Hg.[9,10]

Most studies have been performed in healthy volunteers. Investigations of lymphatic pressure in patients with lymphatic diseases have not been performed because direct measurements are difficult and potentially harmful. Therefore, research into lymphatic pumping during the last few decades provided physicians with little knowledge about how this process is affected by disease or aging. This is unfortunate, because these data would be invaluable in a clinical setting. This stagnation of clinical research is predominantly attributable to the lack of a noninvasive method of measuring lymphatic pumping.

In 2007, however, Modi et al[1] introduced a minimally invasive method of measuring arm lymphatic pressure using lymphoscintigraphy. This technique was applied to healthy individuals and those with lymphedema. Researchers used a standard sphygmomanometer cuff to interrupt lymph flow by inflating the cuff to 60 mm Hg (cuff pressure ranging from 60 to 70 mm Hg had previously been documented as sufficient for preventing lymph flow from the wrist to the axilla).[11] Next, cuff pressure was reduced by 10-mm Hg decrements until it reached 0 mm Hg. The resulting time-activity curves indicated that lymphatic pressure could be defined as the value at which the radiotracer passed under the cuff.

Lymphoscintigraphy is currently considered a major imaging modality for the diagnosis of patients with lymphatic disorders.[12,13] However, this technique is time consuming, expensive, and potentially teratogenic during pregnancy, and, therefore, has only been performed on select patients.[14]

We recently introduced indocyanine green (ICG) fluorescence lymphography as a novel imaging test to visualize superficial (dermal and subdermal lymphatics) real-time lymph flow. Furthermore, we reported that this imaging technique is useful for assessing lymphatic flow both morphologically and functionally.[15-17] In this study, we adapted the ICG fluorescence lymphography technique for measuring superficial lymphatic pumping in the human leg and comparing the contractile force between healthy and lymphedematous legs.

METHODS

This study was approved by the Ethical Committee of Hamamatsu University School of Medicine. Informed consent was obtained from all participants.

Measuring lymphatic pumping with ICG fluorescence lymphography. Using a 27-gauge needle, we subcutaneously injected 0.3 mL of ICG (Diagnogreen 0.5%; Daiichi-Sankyo Pharmatical, Tokyo, Japan) at the dorsum of each participant's foot. Immediately after the injection, fluorescence images of subcutaneous lymphatic drainage were obtained using an infrared camera system (PDE, Hamamatsu Photonics K.K., Hamamatsu, Japan), which activates ICG with emitted light at a wavelength of 760 nm and filters out light with a wavelength <820 nm. The light source for emission of ICG consisted of 760-nm light-emitting diodes, and the detector was a charge-coupled device (CCD) camera. The fluorescence images were continuously observed on the monitor of a laptop computer (LaVie G, Type T; NEC Co, Tokyo, Japan).

Before the ICG was injected, a custom-made transparent sphygmomanometer cuff was wrapped around the lower leg just below the popliteal fossa (Fig 1) and connected to a standard mercury sphygmomanometer.[18] Measurements of lymphatic pumping were performed with the individual supine or sitting. Immediately after the subcutaneous injection of ICG, the transparent cuff was inflated to 60 mm Hg, and then gradually deflated to lower the pressure by 10-mm Hg steps every 5 minutes. This proceeded until the fluorescence contrast agent exceeded the upper border of the cuff, indicating that lymphatic contraction had overcome the cuff pressure. The value of the cuff pressure at this point was used as a measure of lymph pumping pressure (P_{pump}; Fig 2).

ICG fluorescence lymphography and dynamic lymphoscintigraphy measurement of lymphatic pumping in patients without lymphedema. Between December 11, 2008, and September 18, 2009, we measured lym-

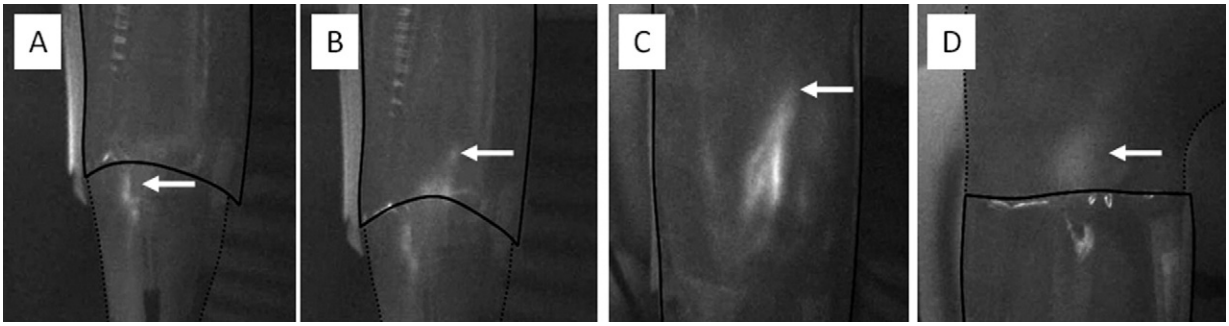

Fig 2. Indocyanine green fluorescence lymphography: **(A)** real-time images of stagnant lymph propulsion at the lower border of the cuff; **(B)** lymph propulsion moving across the lower border of the cuff; **(C)** lymph propulsion moving to the midpoint of the cuff; and **(D)** lymph propulsion moving across the upper border of the cuff. The *arrows* indicate the most advanced indocyanine green contrast agent in the lymph vessel, the *solid line* outlines the cuff, and the *dotted line* outlines the lower leg.

Table. Patient demographics and examination characteristics

Variable	*AAA patients*	*Secondary lymphedema patients*	*Healthy volunteers*
Patients, No.	27	23	15
Legs examined, No.	54	30	30
Examination	ICG lymphography and dynamic lymphoscintigraphy	ICG lymphography	ICG lymphography
Position	Supine	Sitting	Sitting
Gender, No.			
Male	21	0	0
Female	6	23	15
Age, mean ± SD, y	71.9 ± 12.1	61.8 ± 11.7	58.5 ± 13.5

AAA, Abdominal aortic aneurysm; *ICG,* indocyanine green; *SD,* standard deviation.

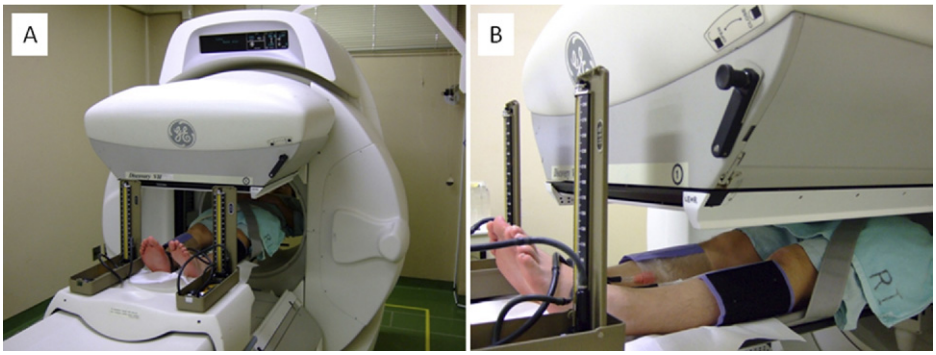

Fig 3. Measuring lymphatic pumping with lymphoscintigraphy. Lymphatic pumping was measured with the individual supine under the gamma camera. Sphygmomanometer cuffs were wrapped around the bilateral lower legs.

phatic pumping in 27 patients (21 men, 6 women) without lymphedema. Patients were a mean age of 71.9 ± 12.1 years and were hospitalized in our surgical ward for treatment of abdominal aortic aneurysms (AAA) and agreed to participate in this study (Table). All study participants were screened for venous insufficiency and deep venous thrombosis by duplex scanning and underwent both ICG fluorescence lymphography and lymphoscintigraphy before AAA surgery.

We collected two pressure readings from both legs of the 27 individuals while they were supine (Fig 1, *A*). The first measurement was taken using ICG fluorescence lymphography; within a couple of days, a second measurement was taken using dynamic lymphoscintigraphy (Fig 3). With this technique, 0.3 mL of technetium 99m-labeled human serum albumin diethylenetriamine pentaacetic acid (activity 111 MBq; Nihon Medi-Physics Co. Ltd., Nishinomiya, Japan) was injected subcutaneously at the dorsum of the

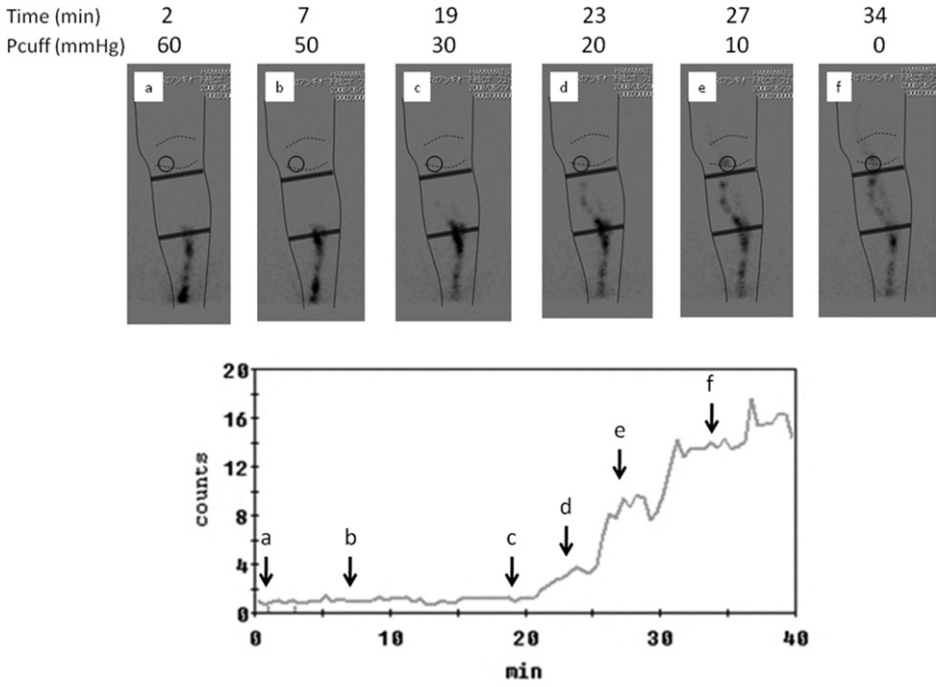

Fig 4. Typical case of a dynamic lymphoscintigraph of the lower leg. The *circles* indicate the site at the upper border of the cuff where the time-activity curves were created. At t = 23 min, with pressure of the cuff (P_{cuff}) at 20 mm Hg, the time-activity curve at the upper border of the cuff shows an elevation in number of counts per minute (*arrow d*), indicating that the lymph pumping pressure is 20 to 29 mm Hg. Panels **a** through **f** (top) correspond with *arrows* **a** through **f** in the graph.

foot, 1 cm from the site at which ICG was previously injected. Because of the gamma camera limitations, dynamic lymphoscintigraphy can only be performed while patients are supine; thus, all individuals remained supine while their legs were imaged. A gamma camera, the Dual-Head Variable-Geometry Nuclear Imaging System (Millenium VG, GE Heatlhcare, Chalfont St. Giles, United Kingdom) was used to obtain images of the lower leg, including the cuff region, every 30 seconds for a period of 40 minutes. These images were used to create time–activity curves of the upper border of the cuff (Fig 4). Lymphatic pumping was measured using the same techniques used for the ICG fluorescence lymphography measurement. Lymphatic pumping pressure was measured as the point at which the lymphatic contraction overcame the cuff pressure, when the counts at the upper border of the cuff steadily rose.

ICG fluorescence lymphography measurement of lymphatic pumping in healthy volunteers and patients with secondary lymphedema. We also wished to compare lymphatic pressure readings in healthy and lymphedematous patients. In both groups, lymphatic pumping of the lower legs was measured while participants remained sitting (Fig 1, *B*) To this end, we examined both legs of 15 healthy female volunteers (mean age, 61.8 ± 11.7 years) and 23 female patients with secondary lymphedema (mean age, 58.5 ± 13.5 years) who came to our vascular surgery outpatients clinic between February 7, 2008, and September 11, 2009 (Table). Lymphedema patients with active bacterial cellulitis were excluded. None of the healthy volunteers had a history of leg edema. Duplex scanning was performed to exclude individuals with deep venous thrombosis or chronic venous insufficiency.

Of the patients with secondary lymphedema, 16 had unilateral leg lymphedema and 7 had bilateral leg lymphedema; all diagnoses were based on lymphoscintigrams performed at our outpatient clinic. All 23 secondary lymphedema patients only underwent compression therapy after manifestation of leg swelling. Five of the 23 patients were diagnosed with stage I lymphedema (mild, easily reversible edema unaccompanied by skin change, sepsis, and limitation of daily activity, and permitting good quality of life with minimal limitations on exercise), and 18 were diagnosed with stage II (moderate edema that is reversible by massage and compression, accompanied by minimal skin change without dermatofibrosclerosis and by occasional sepsis, permitting daily activity with occasional limitations, and allowing fair quality of life with moderate physical limitations).[19] Of the patients with secondary lymphedema, 20 had uterine cancer, 2 had ovarian cancer, and 1 had myoma uteri. Hysterectomy was performed with extended lymph node dissection and local radiation therapy in 13 patients, with lymph node dissection without radiation was in 7, and with no accompanying procedure in 3. There was no difference in lymphatic pumping among the lymphed-

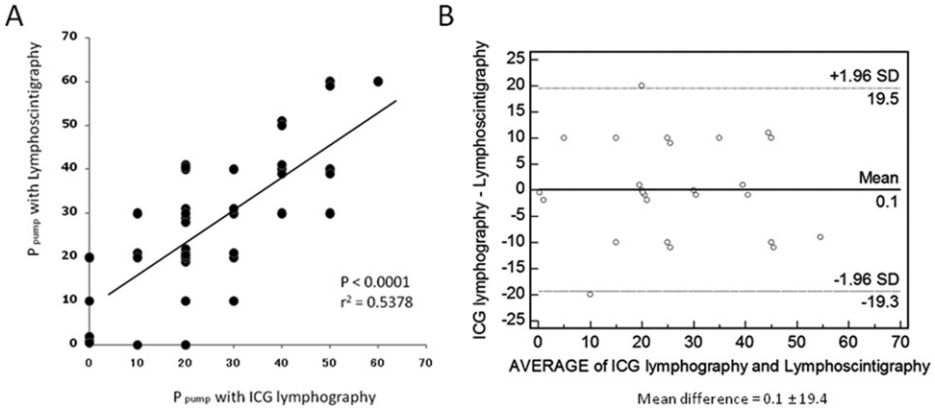

Fig 5. A, Correlation and **(B)** Bland-Altman Plots describe the relationship between the lymphatic pumping obtained by indocyanine green *(ICG)* fluorescence lymphography and dynamic lymphoscintigraphy. **B**, The Bland-Altman diagram shows the mean differences *(solid line)*, and tolerance upper and lower 1.96 ± SD limits *(dotted lines)*. P_{pump}, Lymphatic pumping pressure.

ema patients who had undergone lymph node excision (plus hysterectomy) with radiation, lymph node excision without radiation, or no accompanying procedure (data not shown). All measurements were collected at about noon, before lunch.

Statistical analysis. All data are expressed as mean ± SD, and differences in the means of the two comparison groups were assessed using paired *t* tests. Prism 5 software (GraphPad Software, San Diego, Calif) was used to conduct all statistical analyses, including regressions and correlations. Medicalc 11.2 software (Medcalc Software, Mariakerke, Belgium) was used to perform Bland-Altman analysis to further evaluate the agreement between lymphatic pumping data obtained by ICG fluorescence lymphography and dynamic lymphoscintigraphy. Significance was defined as $P < .05$.

RESULTS

Comparison of lymphatic pumping measurements taken using two different imaging methods. Both measurements were performed while individuals were supine. In the ICG fluorescence lymphography method, real-time images of superficial lymph vessels showed interruption of ICG movement at the lower border of the cuff at 60 mm Hg. Stepwise reduction of cuff pressure restarted the lymph propulsion, and the contrast agent was seen to move across the upper border of the cuff when the lymphatic pumping pressure overcame cuff pressure. This point, P_{pump}, was measured as 25.2 ± 16.7 mm Hg by ICG fluorescence lymphography and as 26.4 ± 16.5 mm Hg by dynamic lymphoscintigraphy (Fig 4). The P_{pump} values were not significantly different between the two groups ($P > .05$; Fig 5). These values were significantly correlated with values taken using dynamic lymphoscintigraphy ($r^2 = 0.54$, $P < .01$). However, Bland-Altman plots showed that two SDs of the mean were approximately 20 mm Hg, suggesting a substantial disagreement between the two methods.

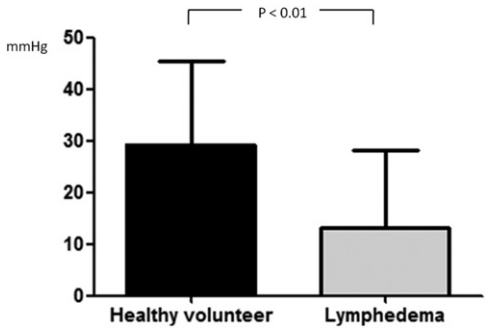

Fig 6. Comparison of lymphatic pumping in healthy legs and lymphedematous legs while volunteers were sitting. Data are shown as mean ± SD.

Comparison of lymphatic pumping in healthy volunteers and patients with secondary lymphedema. ICG fluorescence lymphography was performed while patients were seated. Among healthy individuals, P_{pump} was 29.3 ± 16.0 mm Hg; among those with lymphedema, P_{pump} was 13.2 ± 14.9 mm Hg. These values were significantly different between the two groups ($P < .01$; Fig 6).

DISCUSSION

In this study, we combined the lymphoscintigraphy cuff technique with ICG fluorescence lymphography. Unlike previous methods, our technique enables real-time measurement of lymphatic pressure. We found that 60 mm Hg was enough to stop the superficial leg lymph flow in all participants. Ideally, we would have liked to confirm the accuracy of the ICG fluorescence lymphography values by comparing them with readings obtained from direct cannulation of a pressure transducer into the superficial lymphatic vessel. Because this technique was determined to be invasive and unethical, we used dynamic lymphoscintigram,

which uses the same cuff technique, to provide comparison values of lymphatic pumping. Pressure readings obtained from both techniques were highly correlated. However, values measured with the different techniques differed by as much as 30 mm Hg, indicating that further work will be needed to confirm the accuracy of the new method. The transparent cuff permits us to accurately trace and measure the contrast agent's transit time. In most individuals, this took 3 minutes or less. However, we used time intervals of 5 minutes in case the frequency of the lymphatic contraction might decrease or become stagnant under the cuff pressure.

Lymphatic pumping in healthy legs in AAA patients was determined to be 25.2 ± 16.7 mm Hg while they were supine and 29.3 ± 16.0 mm Hg in healthy volunteers who were sitting. These values were slightly lower than those reported by Olszewski et al.[8] These differences might be caused by our protocol of reducing cuff pressure in 10-mm Hg steps over the course of the reading. This could cause our data to be anywhere from 0 to 9 mm Hg lower than actual pressure values or cause considerable variation among values in each group. In the future, reducing the cuff pressure with smaller steps would likely yield more accurate readings. The variation might also be attributable to the diversity of ages among our participants. One recent study demonstrated that the pumping activity of thoracic ducts was lower in aged rats than in younger rats; therefore, aging might be a critical factor influencing lymphatic pumping values.[20]

Nonetheless, our method has many advantages. First of all, it is easy and safe (ICG is approved for use in hospitals worldwide). It is also less invasive than lymphoscintigraphy and, especially, direct cannulation of a pressure transducer. In addition, ICG fluorescence lymphography is more economical than lymphoscintigraphy and allows measurement of lymphatic pumping in sitting and standing positions. This is particularly important when investigating lymphatic activity in swollen legs, which could be caused by an inability of the lymphatic pumping system to overcome gravitational forces. Although our technique is capable of measuring lymphatic pumping in this position, the bulky gamma camera setting used in lymphoscintigraphy requires that patients remain supine.

On the whole, these many benefits of ICG fluorescence lymphography suggest that it could easily become an office-based diagnostic tool for assessing lymphatic function of the extremities. However, the current technique can only detect lymphatic vessels located ≤2 cm from the body surface; this makes it difficult to detect deep lymph vessels, which run in the subfascial space. Advances in fluorescence contrast agent sensitivity, as well as improvements to imaging instruments, will only serve to enhance the advantages of the ICG fluorescence lymphography technique.[21,22]

In this study, we demonstrated that lymphatic pumping force was reduced in legs with relatively early stages (grade I or II) of secondary lymphedema. This could cause lymph stasis, leading to swelling of the legs. Similar trends have been observed in patients who acquired secondary lymphedema in their arms after undergoing axillary surgery for breast cancer.[1,2] Unfortunately, the mechanisms leading to the reductions in lymphatic pumping reported in these patients still need to be elucidated. Most of the lymphedema patients in our study underwent extended lymph node dissection, which damaged lymphatic passageways and might have increased lymphatic afterload. In animal models of secondary lymphedema, extended lymph node dissection at the inguinal region, together with radiation, caused a high frequency of leg lymphedema.[23] Sustained lymph overload may adversely affect the pump function and overwhelm the lymphatic systems.[24-26] With ICG fluorescence lymphography, we were only able to observe subcutaneous lymph collector vessels and precollector vessels in the fibrous layer of the dermis due to the detection depth of the infrared camera system.

In chronic lymphedema, lymph collectors and also larger lymph vessels are marked by two changes: hyperplasia of the muscle fibers and ill-defined increases in fiber content. Both changes result in dilation of vessels. The increased fiber content also leads to reduced contractibility of the vessels.[27]

Future studies should attempt to measure lymphatic pumping among patients who require axillary or pelvic surgery. Through comparisons of lymphatic pumping before and after surgery, data from this research might enable us to better predict the occurrence of lymphedema in at-risk patients or even diagnose a grade 0 lymphedema, which is currently difficult to diagnose. Furthermore, hitherto unmeasured reductions in lymphatic pumping force may be responsible for diagnoses of "leg edema due to unknown causes." Studies involving ICG fluorescence lymphography will be able to investigate this possibility. Thus, the methods presented in this study are clearly an important first step towards understanding how an increased knowledge of lymphatic pumping can be used in a clinical setting. However, further studies are needed to accumulate data that provide more detailed information on the normal ranges of lymphatic pumping. For instance, measurements should be taken across a larger study population so that comparisons can be made among age groups and between genders. It will also be interesting to measure the response of lymphatic pumping to circadian changes.

CONCLUSIONS

This novel technique of measuring lymphatic pumping in legs using ICG fluorescence lymphography and a transparent sphygmomanometer cuff is safe and minimally invasive, which allows its repeated use. By comparing lymphatic pumping values between healthy and lymphedematous legs, we determined that the contractile force of the lymphatics is reduced in the lymphedematous legs. This suggests that further research with this technique could have important clinical applications. Further, use of this technique may facilitate an improved understanding of how lymph function may contribute to hitherto unexplained cases of leg swelling.

AUTHOR CONTRIBUTIONS

Conception and design: NU
Analysis and interpretation: NU, MN, MS, HT, NY, DS, YM, HK
Data collection: MN, MS, HT, NY, DS, YM
Writing the article: NU
Critical revision of the article: NU
Final approval of the article: NU, MN, MS, HT, NY, DS, YM, HK
Statistical analysis: NU
Obtained funding: Not applicable
Overall responsibility: NU

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Submitted Feb 6, 2010; accepted Apr 23, 2010.